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Plasma sFas and sFas Ligand Levels in Patients With Thrombotic Thrombocytopenic Purpura and in Those With Disseminated Intravascular Coagulation

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Fas, a member of the tumor necrosis receptor superfamily, is 36 kD surface protein containing a single transmembrane region and induces apoptosis by Fas–Fas ligand binding. Soluble Fas (sFas) is produced as the form lacking 21 amino acid residues containing the transmembrane domain by alternative splicing. We found that the plasma sFas levels of 33 patients with thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS), 19 patients with disseminated intravascular coagulation (DIC), and 10 non-DIC patients with multiple organ failure (MOF) were significantly higher than those of 21 non-DIC patients without organ failure and those of 25 healthy volunteers. The plasma sFas ligand levels of the TTP/HUS patients, the DIC patients, and the non-DIC patients with MOF were significantly higher than those of the non-DIC patients without organ failure and those of the healthy volunteers. The plasma sFas levels were significantly correlated with the plasma sFas ligand levels in all subjects. The plasma thrombomodulin (TM) levels were increased significantly in the TTP/HUS patients, the DIC patients, and the non-DIC patients with MOF compared with the levels of the non-DIC patients without organ failure and the healthy volunteers. The plasma sFas antigen levels were correlated significantly with the plasma TM levels in all subjects. These findings suggest that the increases of sFas and sFas ligand that cause apoptosis might be related to the vascular endothelial cell injuries in TTP and DIC with organ failure. Am. J. Hematol. 61:21–25, 1999.

Key words: sFas; sFas-ligand; DIC; TTP; apoptosis

INTRODUCTION

Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) have the same fundamental pathologic lesion and symptoms i.e., thrombotic microangiopathy, microangiopathic hemolytic anemia, thrombocytopenia, fever, neurological symptoms, and renal dysfunction induced by many possible causes, all of which initially induce endothelial damage [1–4]. Disseminated intravascular coagulation (DIC) is often associated with severe bleeding tendency and organ failure, and its outcome is poor [5–7]. In hemostatic examinations, a hypercoagulable state has been observed in patients with DIC [8] and in those with TTP [9]. In addition, the plasma thrombomodulin (TM), von Willebrand factor (vWF), tissue type plasminogen activator (t-PA), and plasminogen activator inhibitor-I (PAI-I) levels were found to be significantly increased in DIC patients with organ failure [8] and in patients with TTP [9], suggesting that vascular endothelial cell injuries exist in these patients.

It was reported that the plasma from patients with TTP

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TABLE I. Diagnostic Criteria at DIC*

		DIC score points
1. PT ratio	1.25–1.66	1
	>1.67	2
2. Fibrinogen (g/l)	1.00–1.50	1
	<1.00	2
3. FDP (μg/ml)	10–20	1
	20–40	2
	>40	3
4. Platelet count ($\times 10^4/\mu l$)	<12	1
	5< <8	2
	<5	3
5. Bleeding tendency	(+)	1
6. Organ failure due to thrombosis	(+)	1

*DIC, disseminated intravascular coagulation. The sum of the DIC score in 1, 2, 3, and 6 was four or higher in the nonleukemic group. The sum of the DIC score in 1–6 was seven or higher in leukemic group.

induces apoptosis in microvascular endothelial cells [10], and that the apoptosis induces a hypercoagulable state in vascular endothelial cells [11]. We hypothesized that the vascular endothelial injuries in TTP and DIC are caused by apoptosis induced by several plasma factors and that the apoptosis might activate the making of microthrombi. The Fas/Apo-1 molecule is a 36 kD cell-surface receptor that belongs to the nerve growth factor–tumor necrosis factor-alpha (NGF–TNF-α) receptor family of apoptosis-signaling molecules [12]. Soluble Fas (sFas) is produced as the form lacking 21 amino acid residues containing the transmembrane domain by alternative splicing. sFas is thought to act as an inhibitor of Fas–Fas ligand binding and in the blockade of Fas-mediated apoptosis [13].

In this study, we measured the plasma sFas and Fas ligand levels that cause apoptosis, in patients with TTP, DIC, and organ failure, and we examined the relationships between plasma TM and Fas or Fas ligand levels.

SUBJECTS

We examined 24 patients with acute phase of TTP, 9 with HUS, 19 patients with DIC, 21 non-DIC patients without organ failure, 10 non-DIC patients with multiple organ failure (MOF), and 25 healthy volunteers. The mean age was 46 ± 15 years (female:male = 19:14) in the TTP/HUS group, 60 ± 15 years of age (7:12) in the DIC group, 58 ± 12 years of age (8:13) in the non-DIC without organ failure group, and 61 ± 17 years of age (4:6) in the non-DIC patients with MOF. The underlying diseases of DIC were hematological malignancies (8 patients), solid cancers (6 patients), and sepsis (5 patients); those of non-DIC were hematological malignancies (14 patients), solid cancers (10 patients), and sepsis (7 patients). The diagnosis of DIC was based on a modified version of the criteria established by the Japanese Ministry of Health and Welfare (Table I) [14,15]. Organ failure was considered to have occurred in the lung when the PaO2 was 50 mmHg or less; in the kidney when creatinine was 3 mg/dl or more; when symptoms of shock from heart failure were present; and when the patient was in a coma or responded only to pain [15].

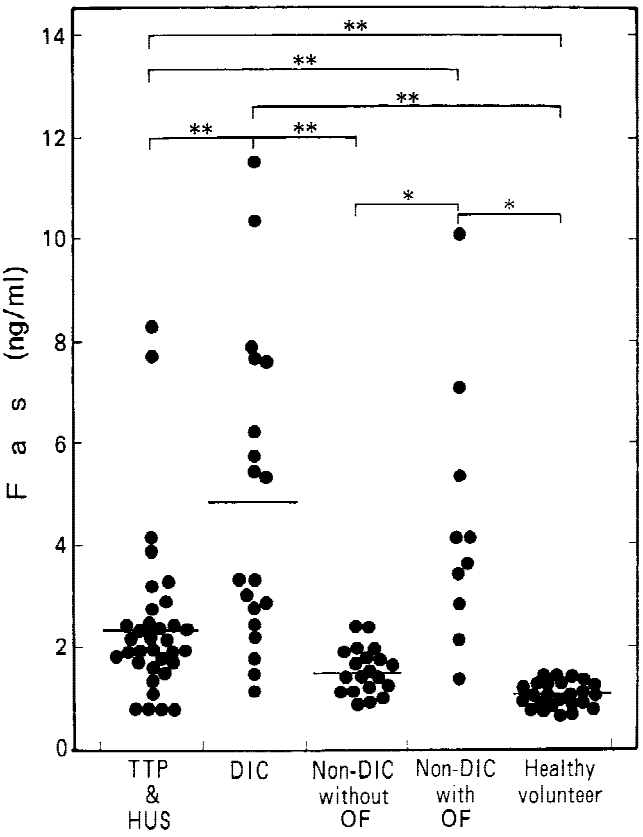

Fig. 1. Plasma sFas antigen levels in patients with TTP/HUS, DIC, non-DIC without organ failure, and non-DIC with organ failure, and healthy volunteers. *, $P < 0.05$; **, $P < 0.01$.

Plasma sFas and sFas ligand antigens were measured by an sFas enzyme-linked immunosorbent assay (ELISA) kit (Medical & Biological Laboratories Co., Nagoya, Japan) and an sFas ligand ELISA kit (Medical & Biological Laboratories), respectively. The sFAS ELISA kit used Fas antibodies against two different epitopes; one of the antibodies is a polyclonal antibody and recognizes the intracellular domain (no. 305-319 a.a.), and the other is a monoclonal antibody that recognizes the extracellular domain (no. 110-120 a.a.) [16]. The sFas ligand ELISA kit used anti-Fas ligand monoclonal antibodies (4H9 and 4A5) against two different epitopes [17]. The plasma TM levels were measured by an ELISA using a thrombomodulin enzyme immunoassay (EIA) kit (Mitsubishi Gas Chemical, Tokyo, Japan).

The data are expressed as mean ± standard deviation (SD). The statistical analyses were performed with the

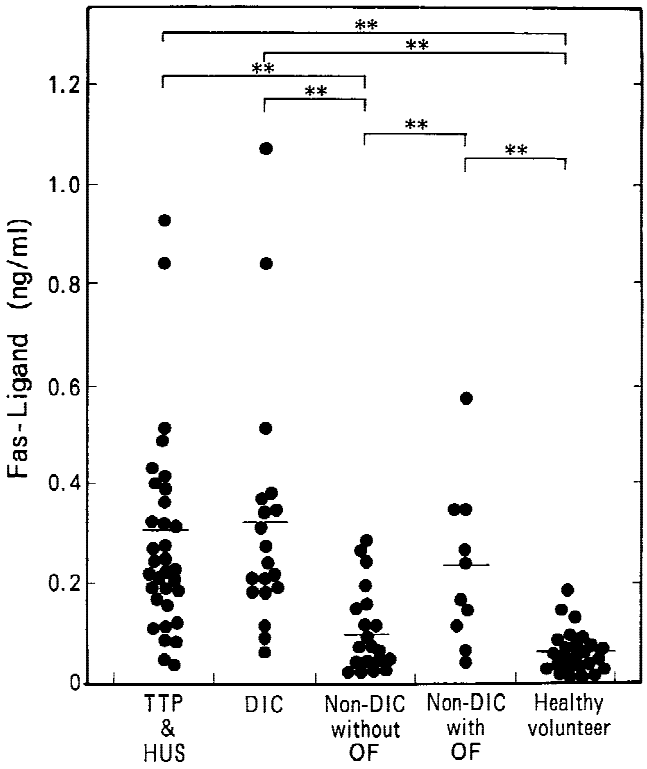

Fig. 2. Plasma sFas ligand antigen levels in patients with TTP/HUS, DIC, non-DIC without organ failure, and non-DIC with organ failure, and healthy volunteers. *, $P < 0.05$; **, $P < 0.01$.

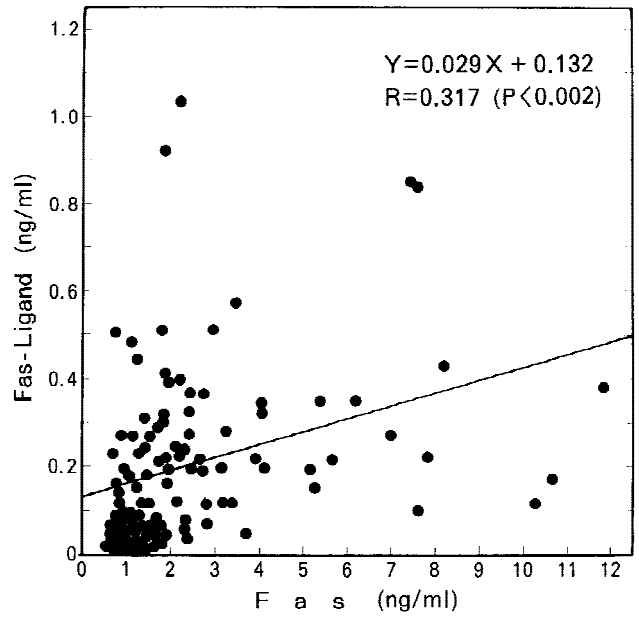

Fig. 3. Correlation between the plasma sFas levels and sFas ligand levels in all subjects.

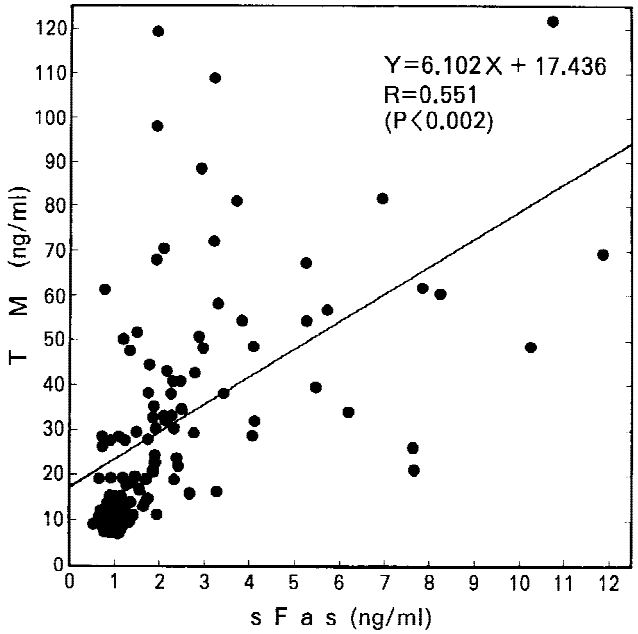

Fig. 4. Correlation between the plasma sFas levels and plasma thrombomodulin levels in all subjects.

TABLE II. Plasma TM Levels in Patients With TTP/HUS, DIC, Non-DIC With MOF, or Non-DIC Without Organ Failure and in Healthy Volunteers*

	TM (ng/ml)	*P* value
TTP/HUS	46.6 ± 26.6	<0.01
DIC	59.4 ± 27.3	<0.01
Non-DIC with MOF	39.3 ± 15.8	<0.01
Non-DIC without organ failure	18.6 ± 6.6	NS
Healthy volunteers	10.5 ± 1.9	

*TM, thrombomodulin; TTP, thrombotic thrombocytopenic purpura; HUS, hemolytic uremic syndrome; DIC, disseminated intravascular coagulation; MOF, multiple organ failure.

Wilcoxon test and Student's *t*-test. *P* values of < 0.05 in both tests were considered significant.

RESULTS

The plasma sFas levels of the TTP/HUS patients (2.39 ± 1.62 ng/ml), and the DIC patients (4.82 ± 3.01 ng/ml), and the non-DIC patients with MOF (4.45 ± 2.57 ng/ml) were significantly higher than those of the healthy volunteers (1.01 ± 0.24 ng/ml) ($P < 0.01$, respectively). The plasma sFas levels of the TTP/HUS patients and DIC patients were significantly higher than those of the non-DIC patients without organ failure (1.56 ± 0.51 ng/ml) ($P < 0.05$, respectively) (Fig. 1). The plasma sFas ligand levels of the TTP/HUS patients (0.307 ± 0.230 ng/ml), the DIC patients (0.321 ± 0.245 ng/ml), and the non-DIC patients with MOF (0.229 ± 0.150 ng/ml) were signifi-

TABLE III. Relationships Among sFas, sFAS Ligand, and TM*

	Fas	Fas ligand	TM
Fas		R = 0.317 ($P < 0.01$)	R = 0.551 ($P < 0.002$)
Fas ligand	R = 0.317 ($P < 0.01$)		R = 290 ($P < 0.02$)
TM	R = 0.551 ($P < 0.002$)	R = 290 ($P < 0.02$)	

*sFas, soluble Fas, TM, thrombomodulin.

cantly higher than those of the healthy volunteers (0.057 ± 0.039 ng/ml) ($P < 0.01$, respectively). The plasma sFas ligand levels of the patients with TTP/HUS and those with DIC were significantly higher than the levels of the non-DIC patients without organ failure (0.084 ± 0.079 ng/ml) ($P < 0.05$, respectively) (Fig. 2). The plasma sFas levels were significantly correlated with the plasma sFas ligand levels in all subjects ($R = 0.317$, $P < 0.002$) (Fig. 3). The plasma TM levels were significantly increased in the TTP/HUS patients (46.6 ± 26.6 ng/ml, $P < 0.01$), the patients with DIC (39.3 ± 15.8 ng/ml, $P < 0.01$), and the non-DIC patients with MOF (56.9 ± 27.7 ng/ml, $P < 0.01$) compared with those of the non-DIC patients without organ failure (18.6 ± 6.6 ng/ml) and the healthy volunteers (10.5 ± 1.9 ng/ml) (Table II). The plasma sFas antigen levels were significantly correlated with the plasma TM levels in all subjects ($R = 0.551$, $P < 0.002$) (Fig. 4, Table III).

DISCUSSION

The reported frequency of TTP in human immunodeficiency virus type-1 (HIV-1) infection, 3.7 per 100,000 [18–20] is almost 40 times that of TTP in the HIV-1-negative population, 0.1 per 100,000 [21]. It is thought that HIV-infected CD4 positive T cells are involved in Fas-mediated apoptosis [22,23]. Plasma from patients with TTP was reported to induce apoptosis in microvascular endothelial cells [10], suggesting that microvascular apoptosis might be caused by several plasma factors in TTP.

In the present study, the plasma sFas levels of the TTP/HUS patients, the DIC patients, and the non-DIC patients with MOF were significantly higher than those of the non-DIC patients without organ failure and the healthy volunteers. The Fas/Apo-1 molecule is a 36 kD cell-surface receptor that belongs to the NGF–TNF-α receptor family of apoptosis-signaling molecules [12]. sFas is produced as the form lacking 21 amino acid residues containing the transmembrane domain by alternative splicing. sFas is thought to act as an inhibitor of Fas–Fas ligand binding and in the blockade of Fas-mediated apoptosis [13]. It was reported that serum Fas levels were elevated in patients with B and T cell leukemia [24] and autoimmune diseases [25]. The plasma sFas ligand levels of the present patients with TTP/HUS, the patients with DIC, and the non-DIC patients with MOF were significantly higher than those of the non-DIC patients without organ failure and the healthy volunteers. In DIC and TTP, Fas might be overexpressed in vascular endothelial cells. The fas ligand is a type II membrane protein that belongs to the TNF-α family, and is expressed in T cells and natural killer (NK) cells and induces apoptosis in Fas-expressing cells [17]. Pathologically, the Fas–Fas ligand system is known to cause hepatitis through the induction of the apoptosis against hepatocytes [26]. Elevated levels of serum-soluble Fas ligand were detected in patients with large granular lymphocytic leukemia, NK cell lymphoma [17], and rheumatic diseases [27]. It was reported that lipopolysaccharide or TNF-α induced disseminated endothelial apoptosis [28].

In the present study, the plasma sFAS levels were significantly correlated with the plasma sFAS ligand levels in all subjects, suggesting that both Fas and Fas ligand were increased in some patients with DIC or TTP with organ failure. The plasma TM levels were increased significantly in the TTP/HUS patients, the DIC patients, and the non-DIC patients with MOF compared with those of the non-DIC patients without organ failure and the healthy volunteers, and the plasma sFas antigen levels were significantly correlated with the plasma TM levels in all subjects. These findings suggest that the increases of sFAS and sFAS ligand might be related to the vascular endothelial cell injuries in TTP and DIC with organ failure.

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